

## WHAT IS CLAIMED IS:

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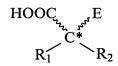
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**/** 1. A method for producing an enantiomerically pure  $\alpha$ -substituted carboxylic acid, said method comprising contacting an aldehyde or ketone with a cyanide containing compound and an ammonia-containing compound or an ammonium salt or an amine, and stereoselectively hydrolyzing the resulting amino nitrile or cyanohydrin intermediate with a nitrilase or a polyheptide having nitrilase activity, wherein the nitrilase is sufficiently active to perform the hydrolysis in the presence of the reaction components, under conditions and for a time sufficient to produce the carboxylic acid.

2. The method according to claim 1, wherein said enantiomerically pure  $\alpha$ substituted carboxylic acid has the following structure:



wherein:

R<sub>1</sub> and R<sub>2</sub> are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, atyl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally R<sub>1</sub> and R<sub>2</sub> are linked to cooperate to form a functional cyclic moiety and

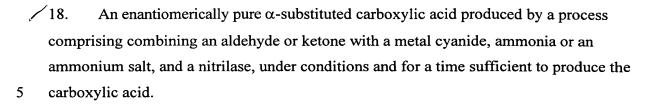
E is  $-N(R_x)_2$  or -OH, wherein each  $R_x$  is -H or lower alkyl.

- 3. The method according to claim 2, wherein said enantiomerically pure  $\alpha$ -substituted carboxylic acid is an  $\alpha$ -amino acid.
- 4. The method according to claim 3, wherein at least one of R<sub>1</sub> and R<sub>2</sub> is substituted or 25 unsubstituted aryl.
  - The method according to claim 4, wherein said enantion erically pure  $\alpha$ -amino 5. acid is D-phenylalanine, D-phenylglycine, or L-methylphenylglycine.

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- 6. The method according to claim 3, wherein said enantiomerically pure  $\alpha$ -amino acid bears a substituted or unsubstituted alkyl side chain.
- 5 7. The method according to claim 6, wherein said enantiomerically pure α-amino acid is L-tert-leucine, D-alanine, or D-hydroxynorleucine.
  - 8. The method according to claim 2, wherein said enantiomerically pure  $\alpha$ -substituted carboxylic acid is an  $\alpha$ -hydroxy acid.
  - 9. The method according to claim 8, wherein at least one of  $R_1$  and  $R_2$  is substituted or unsubstituted aryl.
- The method according to claim 10, wherein said enantiomerically pure α-hydroxy
  acid is (S)-cyclohexylmandelic acid, mandelic acid or 2-chloro mandelic acid.
  - 11. The method according to claim 1, wherein the cyanide is a metal cyanide or a gaseous cyanide.
- 20 12. The method according to claim 11, wherein the cyanide is an alkali cyanide.
  - 13. The method according to claim 11\) wherein the metal cyanide is sodium cyanide.
- 14. The method according to claim 1, wherein the ammonium salt has the formula NH<sub>2</sub>(R)<sub>2</sub><sup>+</sup>X, wherein each R is independently –H or lower alkyl, and X is a counter ion.
  - 15. The method according to claim 14, wherein X is a halide.
  - 16. The method according to claim 15, wherein the halide is Cl.
  - 17. The method according to claim 16, wherein the ammonium salt is NH<sub>4</sub>+Cl.

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19. The enantiomerically pure  $\alpha$ -substituted carboxylic acid according to claim 18, having the structure:

$$\begin{array}{c} \text{HOOC}_{\text{tr},\text{pr}} \text{E} \\ \text{R}_{1} & \text{R}_{2} \end{array}$$

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wherein:

 $R_1$  and  $R_2$  are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally  $R_1$  and  $R_2$  are linked to cooperate to form a functional cyclic moiety, and

E is  $-N(R_x)_2$  or -OH, wherein each  $R_x$  is -H or lower alkyl.

20. The enantiomerically pure  $\alpha$ -substituted carboxylic acid according to claim 19, wherein the carboxylic acid is an  $\alpha$ -amino acid.

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- 21. The enantiomerically pure  $\alpha$ -substituted carboxylic acid according to claim 18, wherein the carboxylic acid is an  $\alpha$ -hydroxy acid.
- 22. The method according to claim 1, wherein the nitrilase has an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4.
- 23. The method according to claim 1, wherein the nitrilase is encoded by a nucleic acid sequence as set forth in SEQ IN NO:1 or SEQ ID NO:3.





- 24. The method according to claim 1, wherein the nitrilase has an amino acid sequence at least 70% identical to the amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4 and has nitrilase activity.
- 5 / 25. A substantially purified polypeptide having an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4 and sequences having at least 70% identity thereto and having nitrilase activity.
- / 26. An isolated nucleic acid sequence encoding an amino acid sequence as set forth in 10 SEQ ID NO:2 or SEQ ID NO:4 and sequences having at least 70% identity thereto and having nitrilase activity, and fragments thereof that hybridize to the nucleic acid sequence.
  - / 27. An isolated nucleic acid sequence as set forth in SEQ ID NO:1.

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- ✓ 28. An isolated nucleic acid sequence as set forth in SEQ ID NO:3.
- /29. A substantially purified polypeptide having an amino acid sequence as set forth in SEQ ID NO:2.

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30. A substantially purified polypeptide having an amino acid sequence as set forth in SEQ ID NO:4.

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